Accurate genome analysis of single cells

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Introduction

Whole genome analysis can be performed by next-generation sequencing (NGS) techniques, microarrays, or parallel real-time PCR addressing multiple genomic regions. These analyses require a minimal amount of genomic DNA in the range of 100 to 1000 ng, which corresponds to 16,000–160,000 cells (e.g., human cells). The use of a high number of cells is not appropriate to analyze single-cell variations of the genome. For analysis of genomic differences between individual cells, accurate replication of the single-cell genome is required. Here, we describe the reliability of single-cell whole genome amplification (WGA) and its application in NGS and real-time PCR. For this analysis, the QIAGEN® REPLI-g® Single Cell Kit was used for multiple-displacement-amplification (MDA) utilizing:

- An optimized formulation of Phi29
- High processivity
- High preamplifying activity

NGS and real-time PCR. For this analysis, the QIAGEN® REPLI-g® Single Cell Kit was used for multiple-displacement-amplification (MDA) utilizing:

- PicoGreen dye. Typically, up to 40 µg of WGA DNA was generated during the replication process.
- The process begins with lysis and denaturation of cells.
- The applications presented here are for molecular biology use. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Method

WGA: Single cells were obtained by picking cells under the microscope (human cells) or by dilution (bacterial cells). Cells were stored in 4 µl PBS until use. After single cells were lysed and DNA was denatured using Buffer C2 REPLI-g Single Cell Kit. QIAGEN®, amplification reagents (REPLI-g sc Reaction Buffer, REPLI-g sc DNA Polymerase) were added. Amplification was performed for 8 hours at 30°C. Yield was determined by double-strand–specific (REPLI-g Single Cell Kit, QIAGEN), amplification reagents (REPLI-g sc Reaction Buffer, REPLI-g sc DNA Polymerase). The process was optimized: Phi29 DNA polymerase (REPLI-g Single Cell Kit) together with two individual

Next-generation sequencing of a single cell

Single Bacillus subtilis cells or 10^4 cells were used for whole genome amplification using the REPLI-g Single Cell Kit. Genome sequencing of the Bacillus subtilis genome was performed on the Illumina MiSeq instrument. The applications presented here are for molecular biology use. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Conclusion

REPLI-g single-cell WGA offers:

- Effective lysis of cells and complete DNA denaturation
- Reliable amplification of the whole genome of single cell
- Optimized strand-displacing REPLI-g DNA Polymerase with proofreading activity
- Increased accuracy during a single-cell WGA
- Minimal amplification bias
- Maximized sequence coverage

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